

WHAT IS CLAIMED IS:

1. A method of assaying for the production or regulation of expression of at least one polypeptide from a linear or circular nucleic acid segment comprising a promoter or putative promoter and an open reading frame or putative open reading frame, the method comprising:

- a) obtaining at least one linear or circular nucleic acid segment comprising a promoter or putative promoter and an open reading frame encoding a peptide or putative open reading frame;
- b) placing the nucleic acid segment under conditions conducive to expression of the polypeptide from the open reading frame; and
- c) assaying for the production or regulation of expression of a polypeptide from the open reading frame or putative open reading frame.

2. The method of claim 1, wherein the nucleic acid segment further comprises a terminator.

3. The method of claim 1, wherein the nucleic acid segment is obtained by synthesis.

4. The method of claim 3, wherein the synthesis comprises non-covalent linkage of the promoter to the open reading frame.

5. The method of claim 4, wherein the non-covalent linkage is performed by:

- a) obtaining a PCR® product comprising the nucleic acid segment, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the promoter can link;
- b) providing a promoter; and

- c) non-covalently linking the promoter to the nucleic acid segment to create the linear or circular expression element.

6. The method of claim 5, further comprising non-covalent linkage of a terminator to the open reading frame.

7. The method of claim 5, wherein the primer that has complementary stretches comprising deoxyuridines comprises the promoter and the terminator in divergent orientation, such that the step of non-covalently linking the promoter and the terminator to the open reading frame results in a circular expression element.

8. The method of claim 1, wherein the nucleic acid segment is obtained by PCR®.

9. The method of claim 1, wherein the nucleic acid segment is a linear nucleic acid that is cut out of a plasmid.

10. The method of claim 1, wherein the nucleic acid segment is placed into a cell.

11. The method of claim 10, wherein the nucleic acid segment is not integrated into the cell's genome.

12. The method of claim 10, wherein the cell is in cell culture.

13. The method of claim 10, wherein the cell is comprised in an organism.

14. The method of claim 13, wherein the organism is a mammal.

15. The method of claim 13, wherein the organism is a plant.

16. The method of claim 10, wherein the linear nucleic acid is injected into the cell.

17. The method of claim 16, wherein said injection comprises microprojectile bombardment.

18. The method of claim 10, wherein the cell is a plant cell.

19. The method of claim 10, wherein the cell is an animal cell.

5 20. The method of claim 1, wherein the nucleic acid segment is placed in a cell-free expression reaction.

21. The method of claim 1, further comprising assaying for expression of the polypeptide.

10 22. The method of claim 21, comprising assaying the expression of a reporter gene product encoded in an open reading frame.

23. The method of claim 1, comprising assaying for function of the promoter or putative promoter.

15 24. The method of claim 23, wherein assaying for function of the promoter or putative promoter comprises determining whether a reporter gene product encoded in an open reading frame is expressed.

25. The method of claim 24, comprising comparing the function of two or more putative promoters.

26. The method of claim 1, wherein the nucleic acid segment is a linear nucleic acid segment.

20 27. The method of claim 1, wherein the nucleic acid segment is a circular nucleic acid segment.

28. A method of analyzing a nucleic acid sequence comprising:
- a) obtaining a nucleic acid segment;
 - b) linking the nucleic acid segment to a promoter and a terminator to create a linear or circular expression element;
 - 5 c) providing the linear or circular expression element to a cell-free expression system or to a cell under conditions conducive to expression of any product encoded for by the nucleic acid segment; and
 - d) analyzing any expression of any product encoded by the nucleic acid sequence.
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29. The method of claim 28, wherein the nucleic acid segment is non-covalently linked to the promoter.
30. The method of claim 29, wherein the non-covalent linkage is performed by:
- a) obtaining a PCR® product comprising the nucleic acid segment, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the promoter can link;
 - 15 b) providing a promoter; and
 - 20 c) non-covalently linking the promoter to the nucleic acid segment to create the linear or circular expression element.
31. The method of claim 30, further comprising non-covalent linkage of a terminator to the open reading frame.
- 25 32. The method of claim 28, wherein the nucleic acid sequence comprises an open reading frame.

33. The method of claim 28, wherein more than one distinct nucleic acid segment is analyzed in a single procedure.

34. The method of claim 28, wherein the linear or circular expression element is placed into a cell.

5 35. The method of claim 34, wherein the linear or circular expression element is not integrated into the cell's genome.

36. The method of claim 34, wherein the cell is comprised in an organism.

37. The method of claim 34, wherein the linear or circular expression element is injected into the cell.

10 38. The method of claim 37, wherein said injection comprises microprojectile bombardment.

39. The method of claim 28, wherein the linear or circular expression element is placed in a cell-free expression reaction.

15 40. A method of analyzing a nucleic acid segment for activity as a promoter comprising:

- a) obtaining a nucleic acid segment encoding a putative promoter;
- b) linking the nucleic acid segment encoding the putative promoter to a nucleic acid encoding a polypeptide to create a linear or circular expression element;
- 20 c) providing the linear or circular expression element to a cell-free expression system or to a cell under conditions conducive to expression of the polypeptide; and
- d) analyzing any expression of the polypeptide.

41. The method of claim 40, wherein the nucleic acid segment encoding the putative promoter is non-covalently linked to the nucleic acid encoding a polypeptide.

42. The method of claim 41, wherein the non-covalent linkage is performed by:

- a) obtaining a PCR® product comprising the nucleic acid segment encoding the putative promoter, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the nucleic acid encoding a polypeptide can link;
- b) providing the nucleic acid encoding a polypeptide; and
- c) non-covalently linking the nucleic acid segment encoding the putative promoter to the nucleic acid encoding a polypeptide to create the linear or circular expression element.

43. The method of claim 40, wherein the nucleic acid encoding the polypeptide is linked to a nucleic acid sequence encoding a terminator.

44. The method of claim 40, wherein the nucleic acid encoding the polypeptide encodes a reporter gene product.

45. The method of claim 44, comprising assaying for expression of the reporter gene product.

46. The method of claim 40, wherein more than one distinct nucleic acid segment encoding a putative promoter analyzed in a single procedure.

47. The method of claim 46, comprising analyzing more than one nucleic acids encoding a putative promoter.

48. The method of claim 46, wherein the nucleic acid encoding a putative promoter is a native nucleic acid.

49. The method of claim 46, wherein the nucleic acid encoding a putative promoter was prepared by mutation of a native promoter sequence.

5 50. The method of claim 40, wherein the linear or circular expression element is placed into a cell.

51. The method of claim 40, wherein the linear or circular expression element is not integrated into the cell's genome.

10 52. The method of claim 50, wherein the linear or circular expression element is injected into the cell.

53. The method of claim 50, wherein the linear or circular expression element is placed in a cell-free expression reaction.

54. A method of screening for a biological response comprising:

- 15 a) obtaining a linear or circular expression element by a process comprising:
obtaining a DNA segment comprising an open reading frame;
linking the open reading frame to a promoter and a terminator to
create a linear or circular expression element; and
- 20 b) providing the expression element to an organism under conditions
conducive to expression of any product encoded for by the open
reading frame.

55. The method of claim 54, wherein the DNA segment is obtained from a process involving PCR®.

56. The method of claim 54, wherein the open reading frame is non-covalently linked to the promoter and the terminator.

57. The method of claim 54, wherein the non-covalent linkage is performed by:

- a) obtaining a PCR® product comprising the open reading frame, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the promoter and terminator can link;
- b) providing a promoter and a terminator; and
- c) non-covalently linking the promoter and the terminator to the open reading frame to create the linear or circular expression element.

58. The method of claim 54, wherein the linear or circular expression element is injected into the organism.

59. The method of claim 54, wherein more than one type of linear or circular expression element is introduced to the organism.

60. The method of claim 54, further defined as a method of producing antibodies for analytical purposes.

61. The method of claim 54, further defined as a method of vaccinating the organism.

62. The method of claim 54, wherein the organism is a mammal or plant.

63. A method of vaccinating an organism comprising:

- a) obtaining a linear or circular expression element by a process comprising obtaining a DNA segment comprising an open reading frame and linking the open reading frame to a promoter and a terminator to create a linear expression element; and

- b) providing the linear expression element to an organism under conditions conducive to expression of any product encoded for by the open reading frame, such that immune response is produced in the animal.

5 64. The method of claim 63, wherein the open reading frame is non-covalently linked to the promoter and the terminator.

65. The method of claim 63, wherein the linear or circular expression element is injected into the organism.

10 66. The method of claim 63, wherein more than one type of linear or circular expression element is introduced to the organism.

67. The method of claim 66, wherein a plurality of types of linear or circular expression elements is introduced to the organism.

68. The method of claim 67, wherein the plurality of types of linear or circular expression elements comprises elements encoding distinct polypeptides of a pathogen.

15 69. The method of claim 68, wherein the pathogen is a virus, bacterium, fungus, alga, protozoan, arthropod, nematode, platyhelminthe, or plant.

70. The method of claim 68, wherein the pathogen is a virus.

20 71. The method of claim 70, wherein individual linear or circular expression elements encoding all potential allergens of a virus is comprised in the plurality of types of linear or circular expression elements.

72. The method of claim 63, wherein the organism is a human.

73. The method of claim 63, wherein the organism is a plant.

74. The method of claim 63, wherein the open reading frame encodes a polypeptide from a cancer.

75. A method of selecting open reading frames effective for generating an immune response specific to a pathogen in an organism, comprising:

- 5 a) preparing a plurality of linear or circular expression elements produced by a method comprising:
- obtaining plurality of DNA segments comprising open reading frames from a pathogen; and
- linking open reading frames to promoters and terminators to create
- 10 a plurality of linear or circular expression elements;
- b) introducing the plurality of linear or circular expression elements into an organism; and
- c) selecting from the plurality of linear or circular expression elements open reading frames that are effective to generate said immune response.

15 76. The method of claim 75, wherein the pathogen is a virus, bacterium, fungus, alga, protozoan, arthropod, nematode, platyhelminthe, or plant.

77. The method of claim 75, further comprising testing said organism against challenge with the pathogen from which plurality of linear or circular expression elements

20 was prepared wherein the organism is protected against challenge with the pathogen.

78. The method of claim 77, wherein one or more antigens conferring a protective response is identified by screening of the organism.

79. The method of claim 75, wherein the plurality of linear or circular expression elements is injected into the organism.

- 25 80. A method of producing a linear or circular expression element comprising:
- a) obtaining a DNA segment comprising an open reading frame; and

- b) linking the open reading frame to a promoter and a terminator to create a linear or circular expression element.

81. The method of claim 80, wherein the DNA segment is obtained from a process involving PCR®.

82. The method of claim 81, wherein the PCR® reaction is primed with oligonucleotides having a complementary stretch incorporating deoxyuridines.

83. The method of claim 82, wherein the deoxyuridines are incorporated every third position of the complementary stretch.

84. The method of claim 80, wherein the open reading frame is non-covalently linked to the promoter and the terminator.

85. The method of claim 84, wherein the non-covalent linkage is performed by:

- a) obtaining a PCR® product comprising the open reading frame, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the promoter and terminator can link;
- b) providing a promoter and a terminator;
- c) non-covalently linking the promoter and the terminator to the open reading frame to create the linear or circular expression element.

86. The method of claim 85, wherein the deoxyuridines are incorporated at every third position of the complementary stretches.

87. The method of claim 85, wherein the primer that has complementary stretches comprising deoxyuridines comprises the promoter and the terminator in divergent

orientation, such that the step of non-covalently linking the promoter and the terminator to the open reading frame results in a circular expression element.

88. A linear or circular expression element produced by a method comprising:

- a) obtaining a DNA segment comprising an open reading frame; and
- b) linking the open reading frame to a promoter and a terminator to create a linear or circular expression element.

89. The expression element of claim 88, wherein the DNA segment is obtained from a process involving PCR®.

90. The expression element of claim 89, wherein the PCR® reaction is primed with oligonucleotides having a complementary stretch incorporating deoxyuridines.

91. The expression element of claim 90, wherein the deoxyuridines are incorporated every third position of the complementary stretch.

92. The expression element of claim 88, wherein the open reading frame is non-covalently linked to the promoter and the terminator.

93. The expression element of claim 92, wherein the non-covalent linkage is performed by:

- a) obtaining a PCR® product comprising the open reading frame, which PCR® product is obtained by amplification from at least one primer that has complementary stretches comprising deoxyuridines with uracil-DNA glycosylase to create overhangs to which the promoter and terminator can link;
- b) providing a promoter and a terminator;
- c) non-covalently linking the promoter and the terminator to the open reading frame to create the linear or circular expression element.

94. The expression element of claim 93, wherein the deoxyuridines are incorporated at every third position of the complementary stretches.

95. The expression element of claim 93, wherein the primer that has complementary stretches comprising deoxyuridines comprises the promoter and the terminator in
5 divergent orientation, such that the step of non-covalently linking the promoter and the terminator to the open reading frame results in a circular expression element.

96. A linear or circular expression element comprising a DNA segment comprising an open reading frame and a promoter and terminator non-covalently linked to said open reading frame.

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